SENSITIVITY OF THE CENTRAL NUCLEI OF THE VAGUS NERVE TO INSULIN DEFICIENCY IN RATS (ENZYME-HISTOCHEMICAL STUDY)

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UDC 616.379:008.64-092.9-07:616.833.191-018.13

The distribution and activity of acetylcholinesterase (AChE) in the central nuclei of the vagus nerve (nucleus dorsalis and nucleus ambiguus) were investigated by Gomori's (1952) histochemical method in sexually mature male rats, intact or with experimental alloxan diabetes. The number of cells with high AChE activity in nucleus dorsalis was increased by 6% in the rats with alloxan diabetes. The results suggest a role of the dorsal nucleus of the vagus nerve in the regulation of pancreatic endocrine function.

KEY WORDS: central nuclei of the vagus nerve; alloxan diabetes; acetylcholinesterase

The vagus nerve is represented in the CNS (at the level of the medulla) by two nuclei – nucleus dorsalis and nucleus ambiguus. In a previous study [1] the authors demonstrated the sensitivity of neurons of the dorsal nucleus of the vagus to insulin deficiency. A karyometric method was used to investigate rats with experimental alloxan diabetes. Because of the small number of neurons which it contains, the nucleus ambiguus could not be studied by this method. This paper described a further investigation of the sensitivity of the central nuclei of the vagus to insulin deficiency in the animal. The reaction of the specific enzyme for cholinergic structures, acetylcholinesterase, was used as the test. Rats with experimental alloxan diabetes were used as a biological model reproducing insulin deficiency.

EXPERIMENTAL METHOD*

Experiments were carried out on noninbred male rats weighing 150-180 g. Alloxan was injected intramuscularly in a dose of 15-20 mg/kg body weight as a 5% aqueous solution. Throughout the experiment the glucose concentration in the urine of the experimental animals was monitored by means of Glucose Test indicator paper. The effectiveness of the alloxan injection was judged from the blood glucose concentration measured by the orthotoluidine method at the time of sacrifice of the animals. The animals were killed 1.5-2 months after injection of the alloxan. The blood glucose concentration in the experimental rats varied from 237 to 540 mg%. Intact male rats of the same weight group, kept under similar conditions on a standard diet, served as the control. Altogether 22 rats with diabetes and 14 intact rats were used.

Tissue samples taken from the region of the medulla from 9-12 experimental and control rats were mounted with solid CO_2 in a single block, from which frontal sections (12 μ) were cut on a cryostat. The reaction for acetylcholinesterase was carried out by Gomori's method [2] at 37°C on unfixed sections for 2 h at pH 6.0. To inhibit nonspecific cholinesterase some of the sections were kept for 30 min in a solution of di-isopropylfluorophosphate (DFP) in a concentration of 10^{-6} M before incubation. After incubation the sections were treated with ammonium sulfide, fixed with 10% neutral formalin, and mounted in balsam.

EXPERIMENTAL RESULTS

In sections through the medulla obtained from the region of the central nuclei of the vagus (Fig. 1) attention was concentrated on the activity and character of distribution of acetylcholinesterase (AChE) in three

*The authors are grateful to A. V. Sakharova for valuable technical assistance in the histochemical part of the investigation.

Laboratory of Experimental Morphology, Institute of Experimental Endocrinology and Hormone Chemistry, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Yudaev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 12, pp. 734-737, December, 1977. Original article submitted May 17, 1977.

 0007-4888/77/8412-1780$07.50

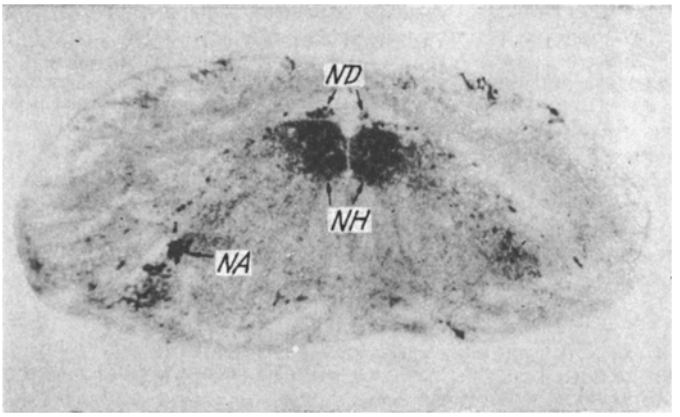

Fig. 1. Acetylcholinesterase in rat medulla at level of central nuclei of vagus nerve. ND) Dorsal nucleus of vagus, NA) nucleus ambiguus, NH) nucleus of hypoglossal nerve, 13 ×.

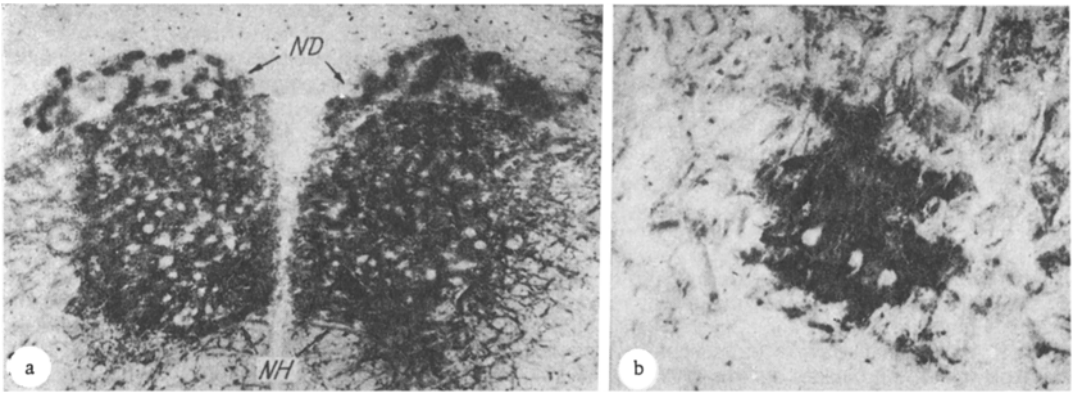

Fig. 2. Distribution of acetylcholinesterase: a: ND) dorsal nucleus of vagus nerve, 37×; NH) nucleus of hypoglossal nerve, 37×; b) nucleus ambiguus, 95×.

groups of motoneurons localized in the region of nucleus dorsalis (ND) and nucleus ambiguus (NA) of the vagus nerve and the nucleus of the hypoglossal nerve (NH).

In the region of ND the enzyme was distributed intracellularly, uniformly throughout the cytoplasm of the neurons; its activity was estimated from the intensity of the staining reaction, which varied from moderate to very high (Fig. 2a). Not only the cell bodies were stained, but also the processes leading from them. Cells with hardly detectable AChE activity also were found.

In the region of NA activity of the enzyme was high: AChE was detected intracellularly but, by contrast with ND, it was distributed irregularly throughout the cytoplasm of the motoneurons, chiefly at the periphery of the cells. The main difference was that the neuropil in NA was intensively stained, evidence that it contains enzyme with high activity (Fig. 2b). The intensively stained neuropil merged with the stained periphery of the cells, so that it was difficult to distinguish the intracellular enzyme individually in the motoneurons of NA and to estimate the level of its activity.

In the region of NH, the AChE activity was lower than in NA. Moreover, the intensively stained neuropil did not merge with the weakly stained periphery of the cells, a fact which distinguished this nucleus from the previous one in its external appearance.

AChE also was detected in the endothelium of the blood vessels. After the use of DFP in a concentration of 10^{-6} M, the reaction for AChE in the capillaries still remained visible (from just detectable to moderate).

Nonspecific cholinesterase (NChE) was detected mainly in the cytoplasm of the cells in ND and in the capillary endothelium. After DFP, no reaction for NChE could be found in the capillaries.

TABLE 1. Number of Nerve Cells with Positive Reaction for Acetylcholinesterase in Dorsal Nucleus of Vagus Nerve of Intact and Experimental Rats, % of Total Number of Cells (M ± m)

Index	Control (5)	Diabetes (11)	P
Cells with negative and weak reaction	52±1,2	53±1,5	—
Cells with moderate reaction	29±1,4	22±0,9	<0,01
Cells with strong reaction	19±1,5	25±1,3	<0,01

Legend. Number of animals investigated shown in parentheses.

In rats with alloxan diabetes AChE activity in the region of NA did not differ appreciably from the intact control; meanwhile, in the region of ND a difference was found in the cholinesterase activity of the motoneurons in the control and experimental animals. For quantitative assessment of visible differences, three groups of cells were counted in sections stained with alum carmine. Group 1 consisted of cells with a negative or weak reaction, group 2 of cells with a moderate reaction, and group 3 of cells with a strong reaction. The counting was carried out on three consecutive sections taken from the same level of the medulla (where the nucleus under investigation was most clearly represented). The total number of cells in each animal was taken as 100%. The number of cells in each group was determined as a percentage of the total number of cells. The results of the counting are given in Table 1. In rats with alloxan diabetes the number of nerve cells with a moderate reaction was 7% lower whereas the number of nerve cells with high activity was 6% greater.

The results correlate with those of the previous investigation [1], in which statistically significant changes in the size of the cell nuclei were found by a karyometric method in ND of rats with experimental alloxan diabetes.

The results of the present investigation confirm the earlier hypothesis regarding the role of cholinergic efferent fibers arising from the dorsal nucleus of the vagus in the nervous regulation of the endocrine function of the pancreas. In view of the rich cholinergic innervation of the other central nucleus of the vagus, NA, the specific distribution of the enzyme in its motoneurons, and the abundance of the enzyme in the neuropil, it was impossible in this investigation to determine the relationship of its motoneurons to this endocrine function on the basis of intracellular AChE activity. To clarify this problem further investigations are needed, including in particular an ultrastructural analysis of the nerve cells of NA under analogous experimental conditions.

LITERATURE CITED

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